EcAMSat and BioSentinel: Autonomous Bio Nanosatellites Addressing Strategic Knowledge Gaps for Manned Spaceflight Beyond LEO

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SUNY CNSE Colloquium

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Ames Research Center

- 1 of 9 NASA Field Centers
- Located in Silicon Valley

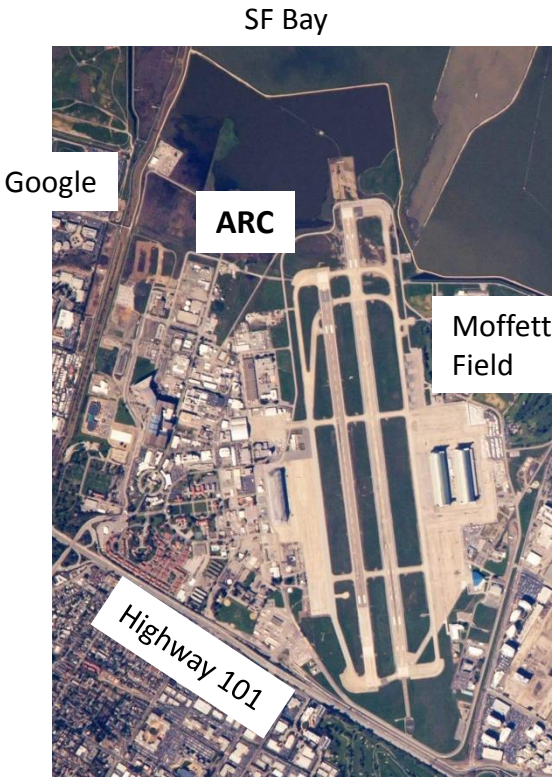


Image taken aboard ISS

- Second oldest NASA facility
  - Est. 1939 as part of National Advisory Committee for Aeronautics (NACA)
  - Absorbed into NASA in 1958
- ~2300 employees

What We Do at Ames

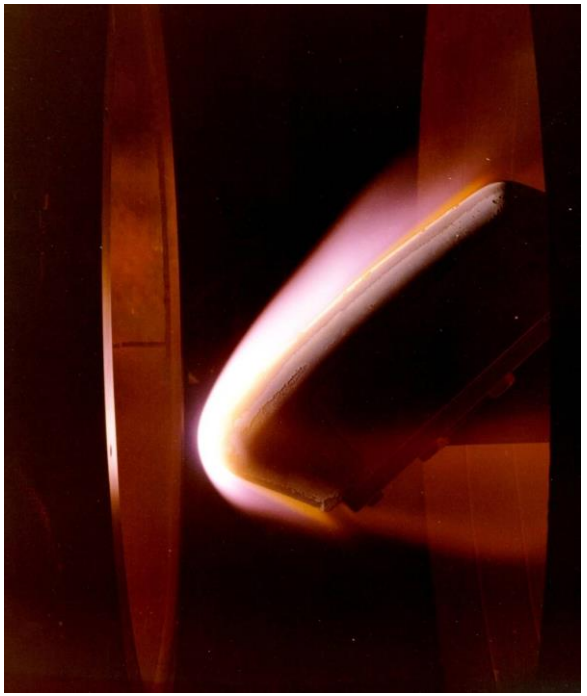
- National Full-Scale Aerodynamics Complex
  - 80x120’ wind tunnel is largest in the world
- Unitary Plan Wind Tunnel
  - 3 test sections, most commercial & military aircraft tested here
  - Mercury, Gemini, Apollo capsules
- NextGen Air Transportation


80x120’ Wind Tunnel


Hangar 1

What We Do at Ames

- ARC Jet
  - Creates super hot plasmas to help simulate re-entry
  - Only facility capable of modeling re-entry from interplanetary space
- Pleiades supercomputer
  - 13th fastest in world
  - Appeared in The Martian!



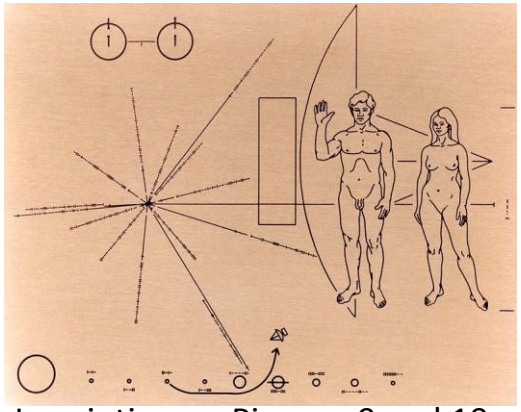
Space shuttle wing tests

What We Do at Ames: Flight Missions

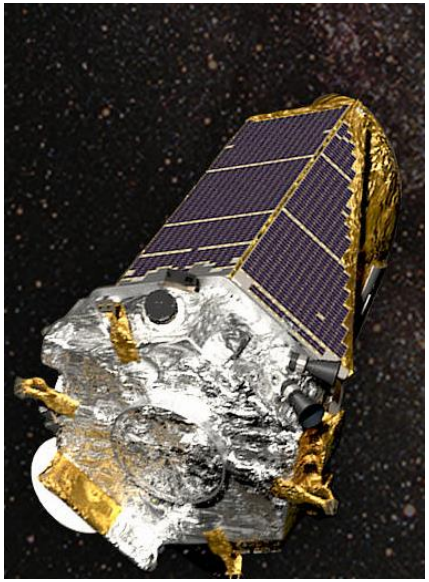
- Pioneer missions (1970s)

- LADEE and LCROSS
  - Measured structure and composition of the thin lunar atmosphere
- Kepler and K2 Missions
  - ~2500 confirmed exoplanets

- ISS Life Sciences
  - Wet-Lab 2, molecular biology in space!
  - Rodent research
  - Fruit fly lab



Inscription on Pioneer 9 and 10

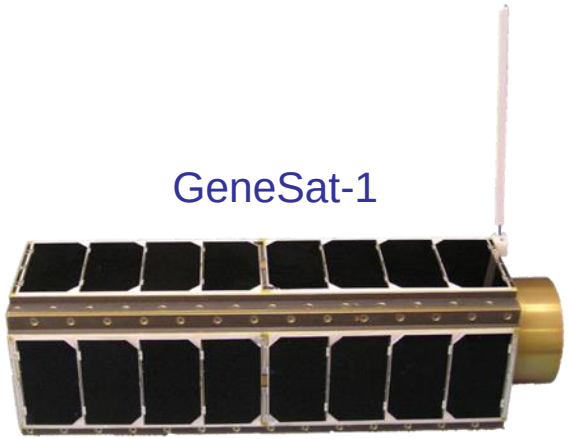


Kepler

Ames Has a History of Successful Bio Nanosats

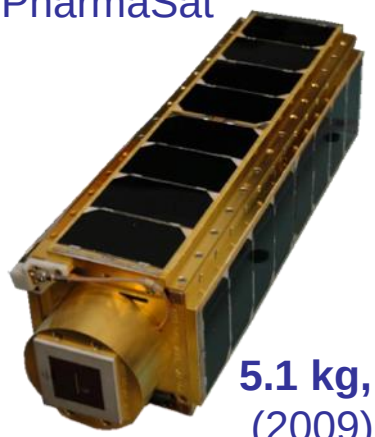
- Nanosats are small, autonomous, free-flying satellites
  - Less reliance on human-tended experiments
  - Access to space: Low-cost launches as secondary payloads
  - Multiple flights possible - test, learn, iterate
  - Excellent education vehicle: Significant academic participation worldwide
  - Peer-reviewed science experiments

GeneSat-1



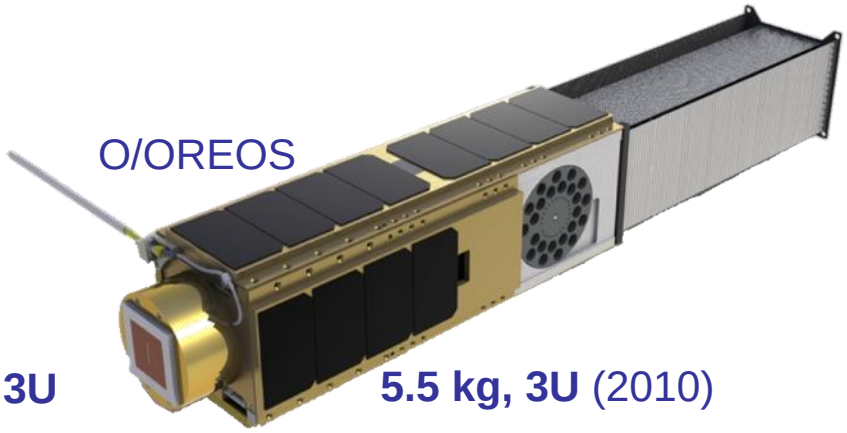
4.4 kg, 3U (2006)

PharmaSat



5.1 kg, 3U (2009)

O/OREOS



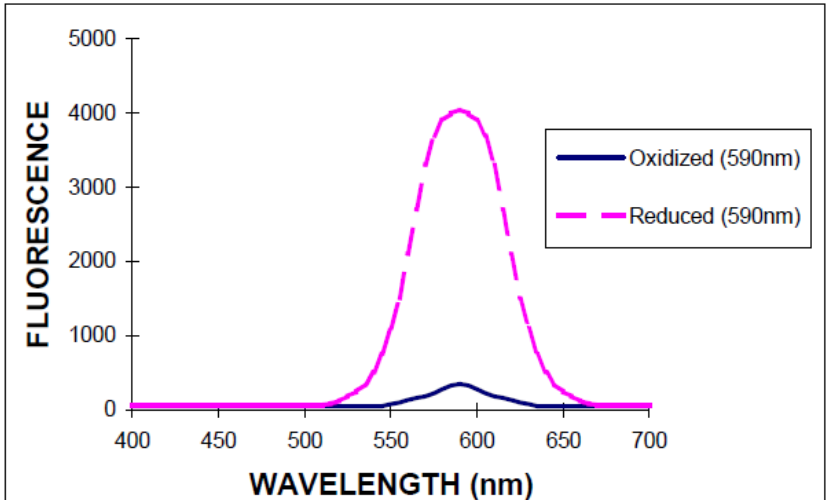
5.5 kg, 3U (2010)

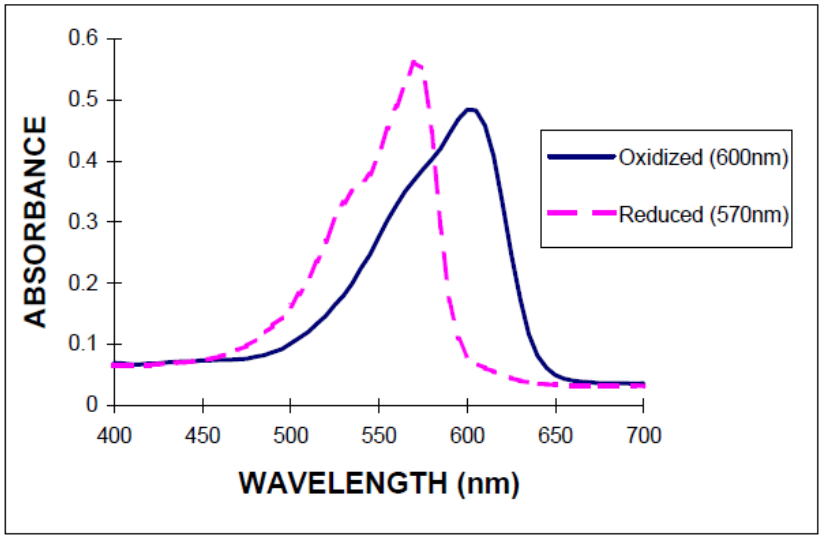
1U = 10x10x10 cm

A Quick Intro to AlamarBlue

- Metabolic indicator dye
  - Reduced by FAD, NADPH, cytochromes
  - Color change from blue to pink
  - Increase in fluorescence
  - Shift in absorbance

- Used by PharmaSat, O/Oreos, EcAMSat and BioSentinel

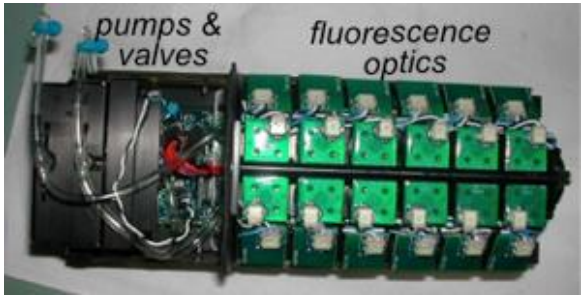


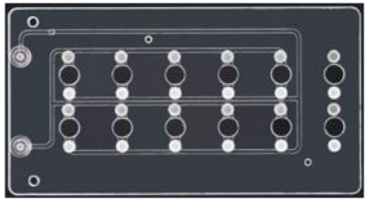


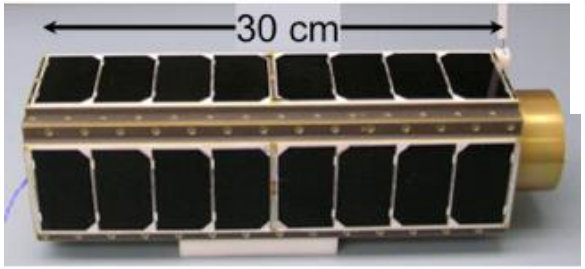
GeneSat-1: 1st biological nanosatellite in Earth orbit, 1st real-time, *in-situ* gene expression measurement in space

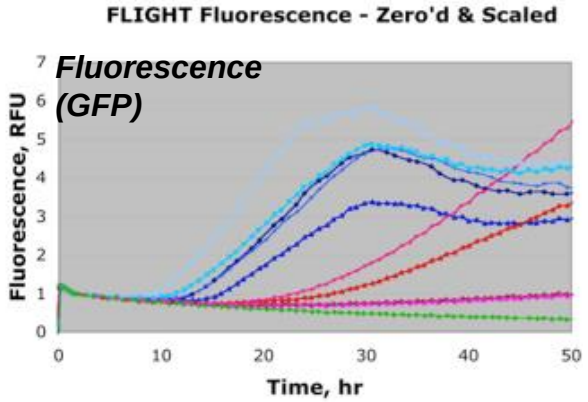
- Launch: December 2006
- Nutrient deprivation in dormant state
- Nutrient delivered upon orbit stabilization
- GFP to measure gene expression
- OD to measure cell population

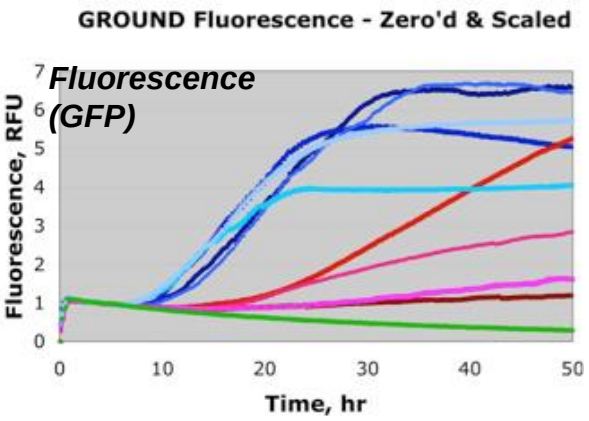
E. coli





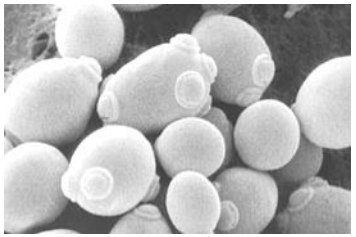




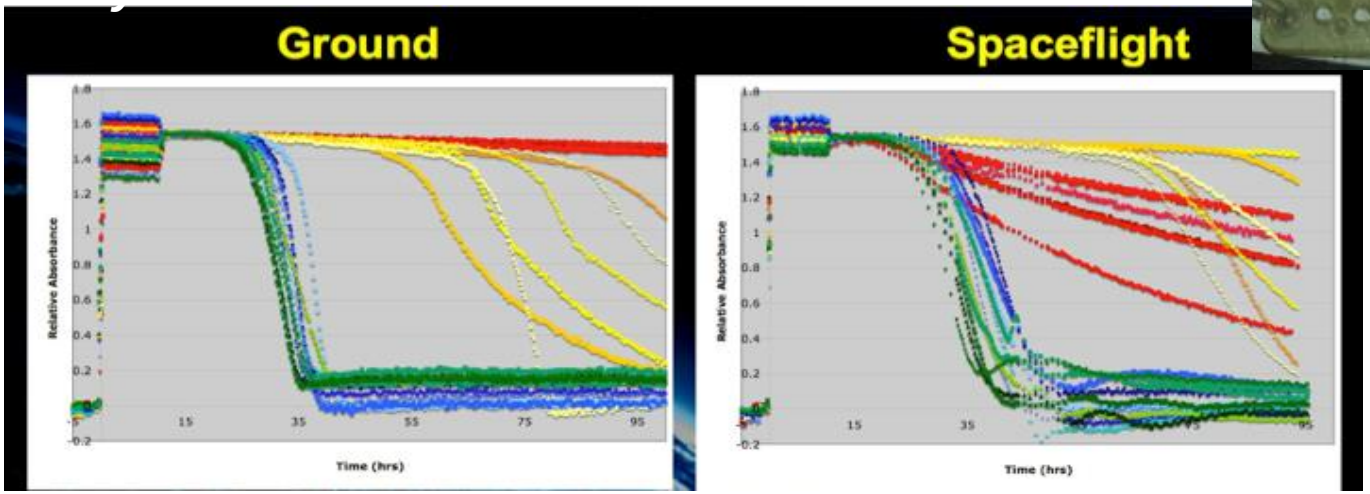


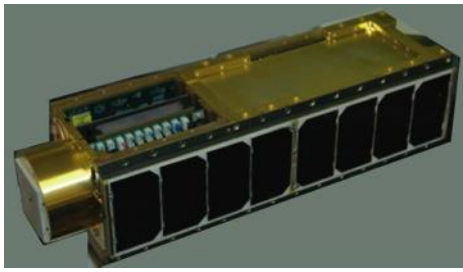
PharmaSat: Effect of Microgravity on Yeast Susceptibility to Antifungal Drugs

- Grow yeast in multiwell fluidics card in µ-gravity
  - Measure inhibition of growth by antifungal
  - Optical absorbance (turbidity: cell density)
  - Metabolism indicator dye: Alamar Blue
  - Control + 3 concentrations of antifungal



S. cerevisiae

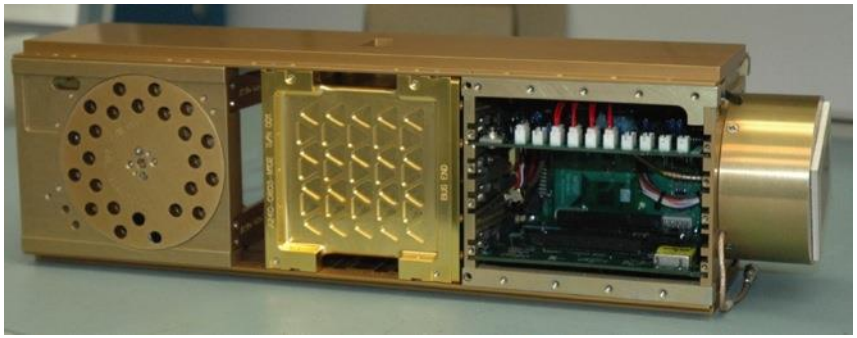


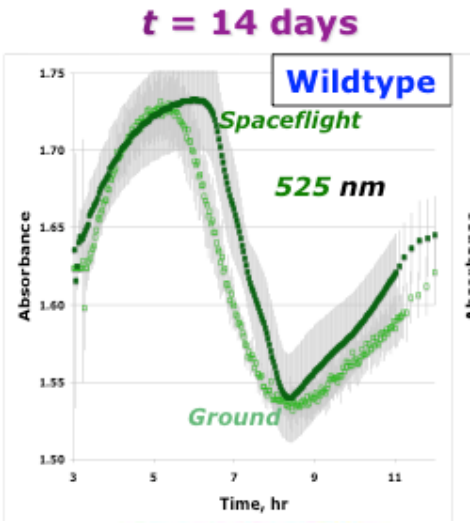


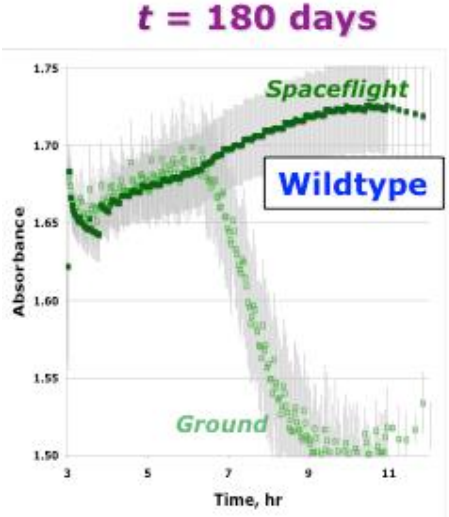
Antonio Ricco, *et* al. Proc. SPIE 7929, Microfluidics, BioMEMS, and Medical Microsystems IX, 79290T (2011)

O/OREOS: Effects of Space exposure on biological organisms (6 mo) and organic molecules (18 mo)

- 2 x 1U payloads
  - SESLO: Monitor survival, growth, metabolism of *Bacillus subtilis: in-situ* OD / colorimetry
  - SEVO Track changes in organic molecules and biomarkers: UV / visible / NIR spectroscopy







O/OREOS: Organism/Organic Response to Orbital Stress
SESLO: Space Environment Survival of Living Organisms
SEVO: Space Environment Viability of Organics

Wayne L. Nicholson, *et al.* Astrobiology, Vol. 11, Issue 10, Dec. 2011, pp. 951-958
Andrew Mattioda, *et al*. Astrobiology, Vol. 12, No 9, 2012
Nathan E. Bramall, *et al*. Planetary and Space Science, Vol. 60, 2012, pp: 121–130
Amanda M. Cook, *et al*. Astrobiology. February 2014, Vol. 14, Issue 2, pp: 87-101

Addressing Strategic Knowledge Gaps

- If humans are to go further into space for longer:
  - Model organisms can help us understand and mitigate key biological risks
    - Direct impact on human health or performance
    - Impact on the biota that accompany humans
    - Impact on organisms used to process waste or produce food

- Deep space radiation
- Long-term exposure to microgravity

EcAMSat

E. coli Anti-Microbial Satellite

EcAMSat Team

- PI: AC Matin, Stanford University
- Stevan Spremo, PM

- Current Team: Matt Chin, Tori Chinn, Aaron Cohen, Charlie Friedericks, Mike Henschke, Chris Kitts, Mike Miller, Mike Padgen, Macarena Parra, Mario Perez, Mike Rasay, Tony Ricco, Tim Snyder

- Support from NASA's Human Exploration and Operations Mission Directorate (HEOMD) as a Small Complete Mission of Opportunity in Fundamental Space Biology.

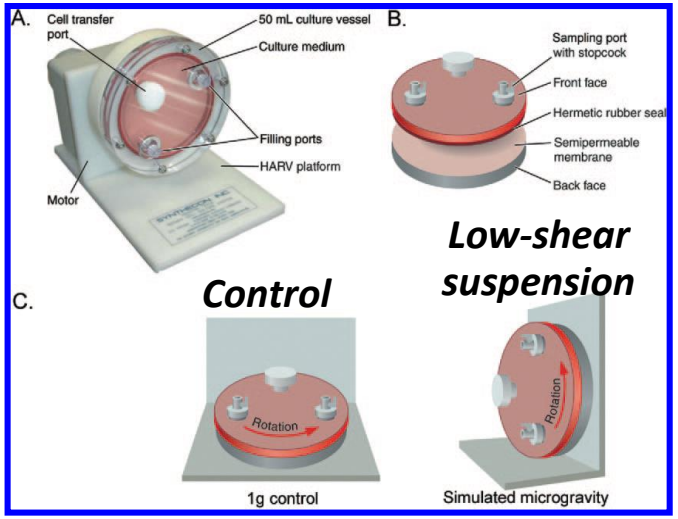
For Astronaut Health During Long Term Spaceflight, Many Questions Remain

- Immediate need to understand antibiotic resistance in microgravity
  - Evidence that bacteria become more virulent and resistant than in normal gravity
  - Astronaut's immune system is compromised in microgravity

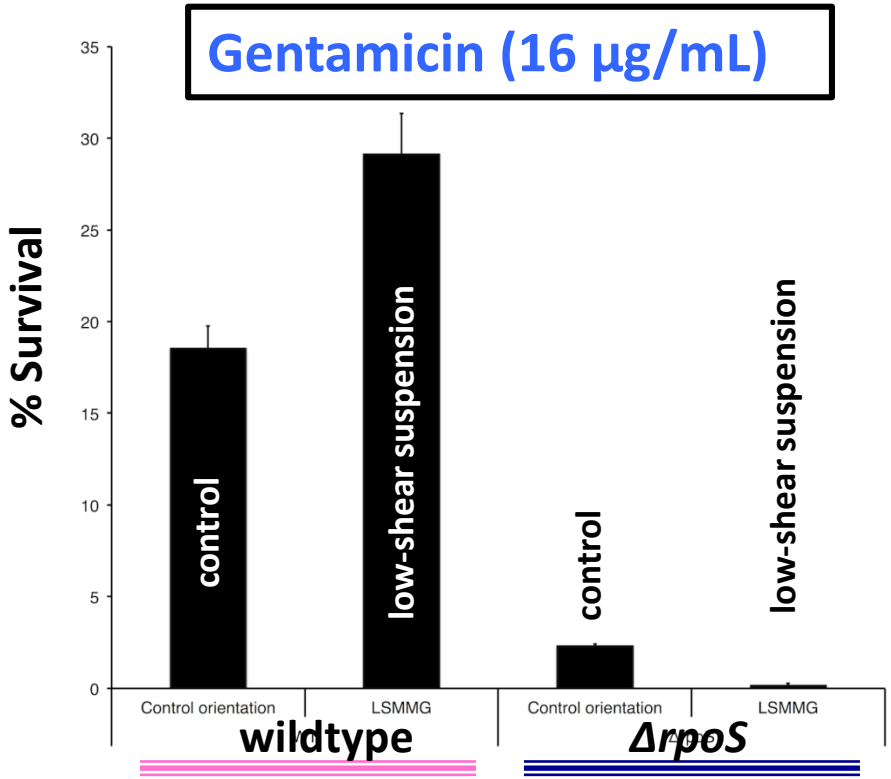
- Model Organism: uropathogenic *Escherichia coli* (UPEC)
  - Urinary tract infections have been reported in astronauts
  - Gentamicin has been used to treat UTI
  - WT and ΔrpoS mutant strains
    - rpoS responsible for activating general stress response (GSR)
    - GSR increases antibiotic resistance

Gentamicin Resistance Increases in Low-Shear Cellular Suspensions

High-aspect-ratio vessels (HARVs)



- ***Control***: rotation about a vertical axis
- ***Low-shear suspension***: rotation about a horizontal axis



- HARVs used to simulate microgravity, although experimental results in actual microgravity can vary

EcAMSat is a Re-flight of PharmaSat Hardware

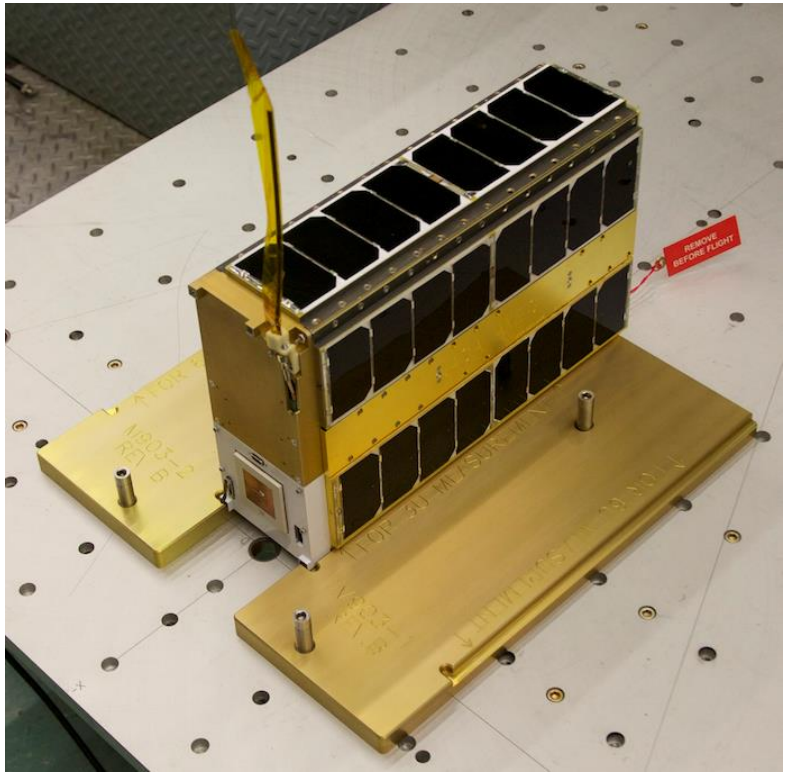
- EcAMSat will measure growth and metabolic activity of *E.* coli treated with antibiotic in microgravity compared to the ground control
- PharmaSat measured growth and metabolic activity of yeast treated with antifungal in microgravity compared to ground control

- Yeast ➔ *E. coli*
  - Filter pore size: 1.2 µm ➔ 0.2 µm
    - Higher pressure in fluidic system
  - Experiment temperature: 27°C ➔ 37°C
    - More power required
  - Storage: dry ➔ wet

EcAMSat is the First* NASA 6U Mission

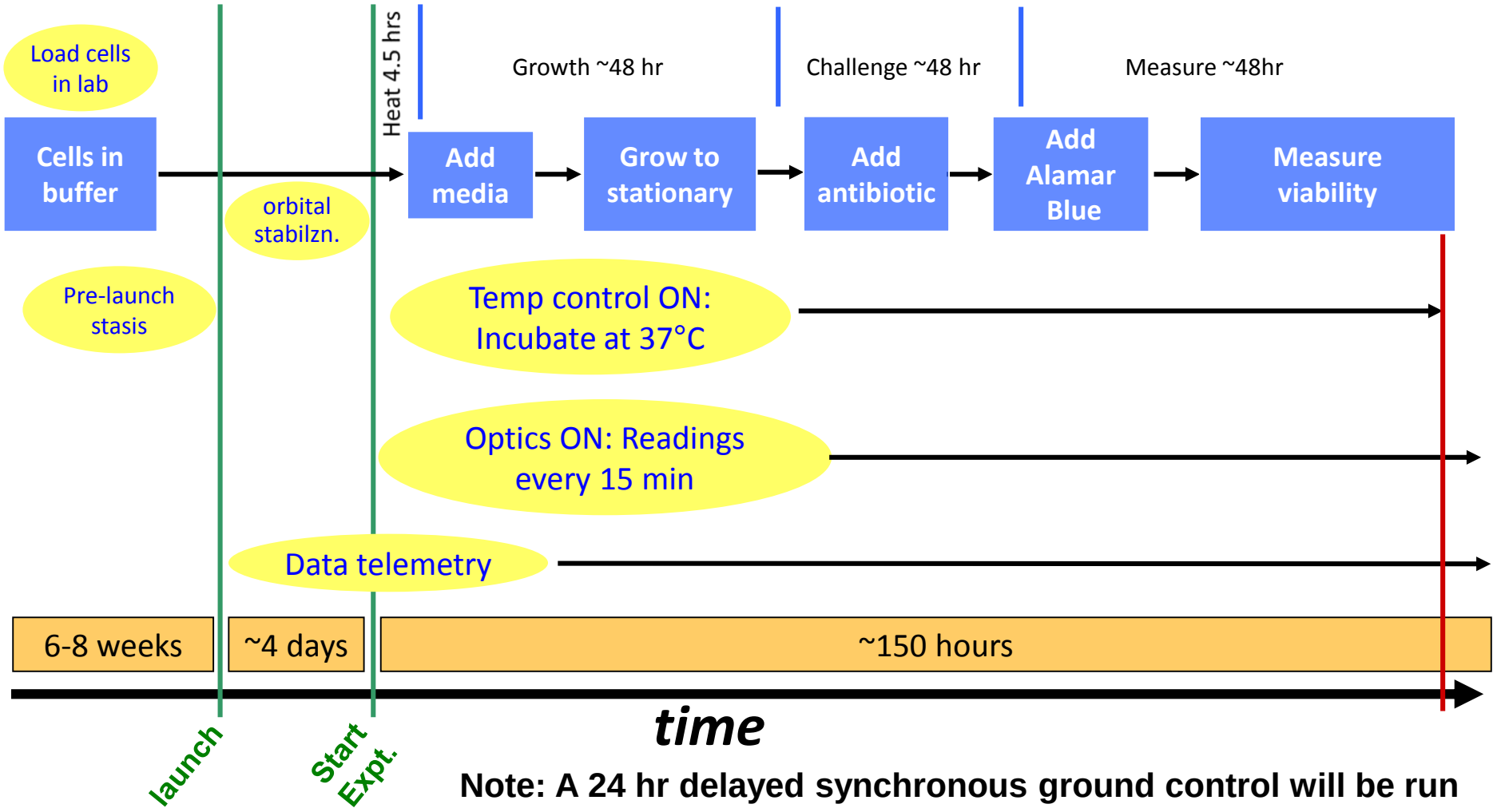
- Overall size: 10 x 22 x 34 cm
- Passive magnetic orientation
- UHF Beacon and S-band radio
- Extensive thermal power management testing and modeling

- Mission Ops run by Santa Clara University



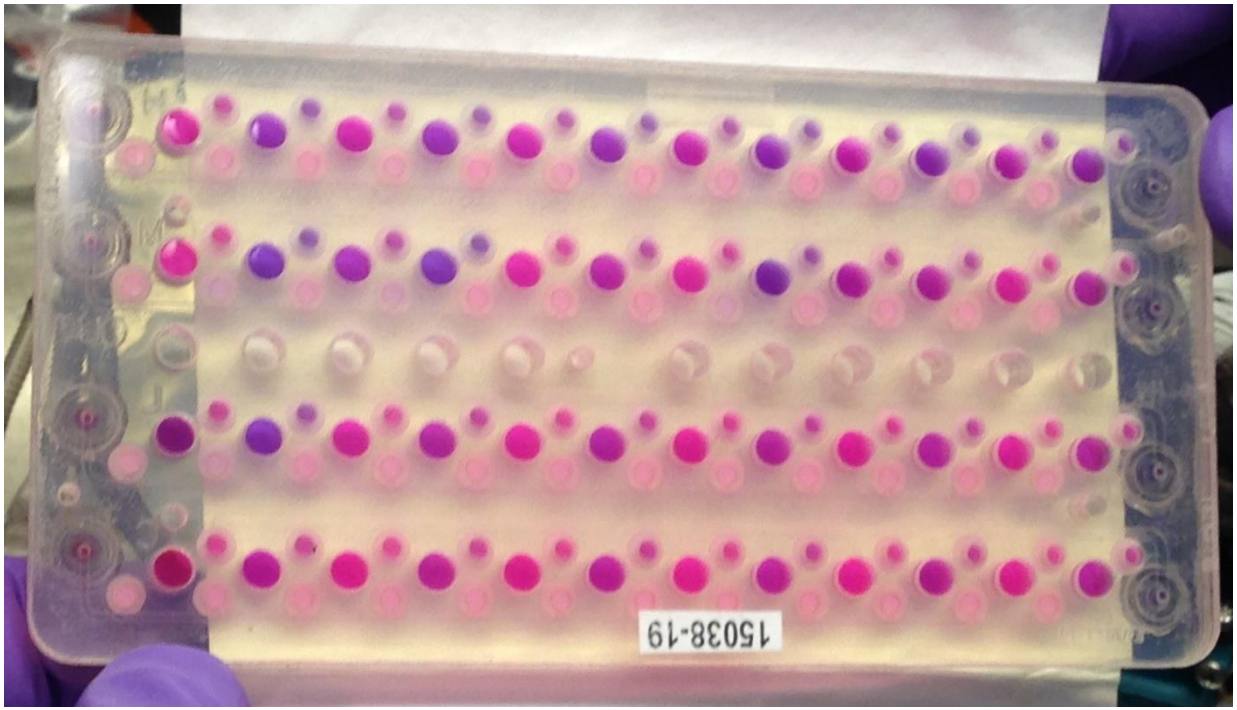
* See slide 24

Experiment Design



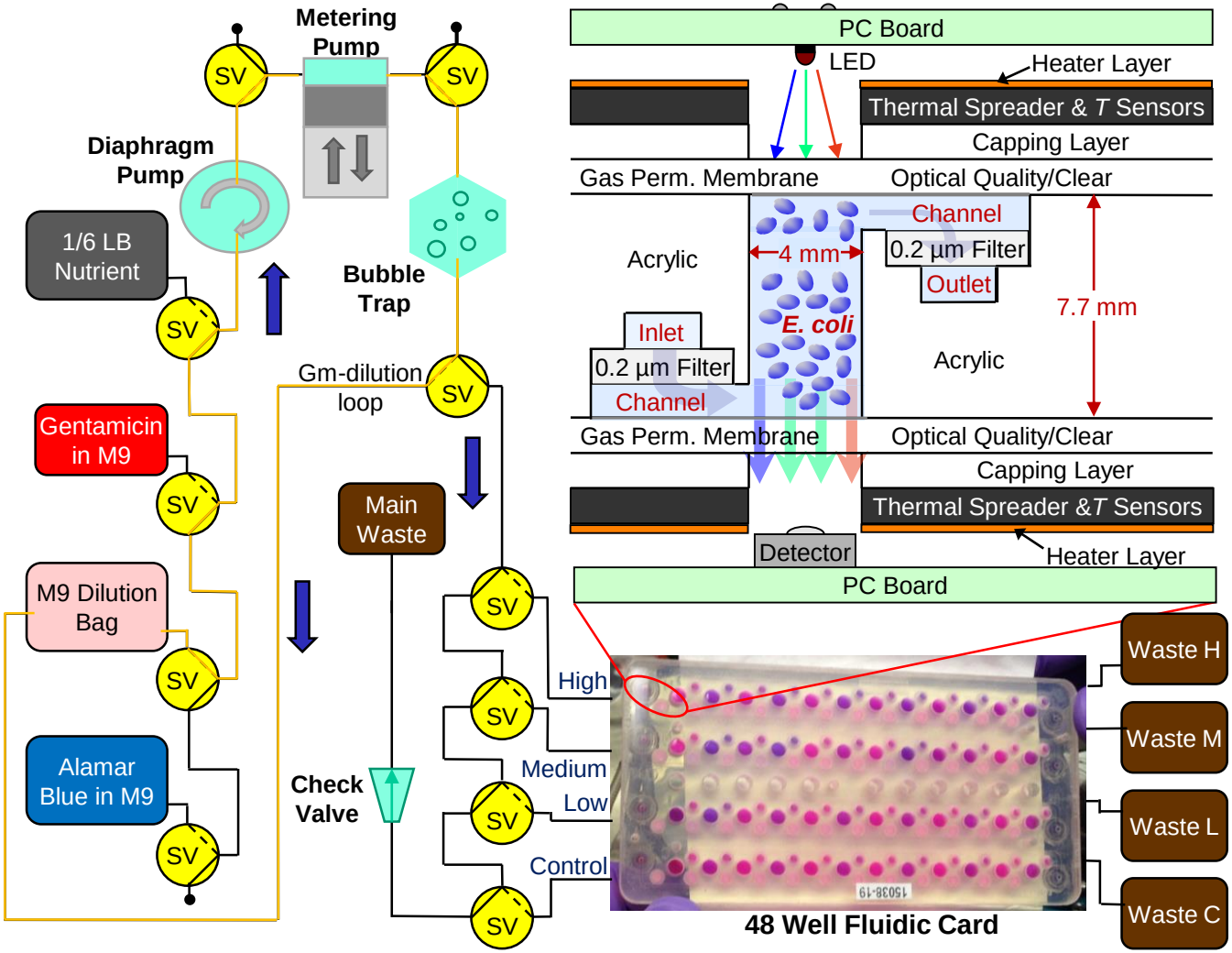
Note: A 24 hr delayed synchronous ground control will be run

EcAMSat Fluidic Card



- WT and mutant strains loaded into alternating wells prior to sealing
- Each bank is independent and will receive a different dose of antibiotic during the experiment

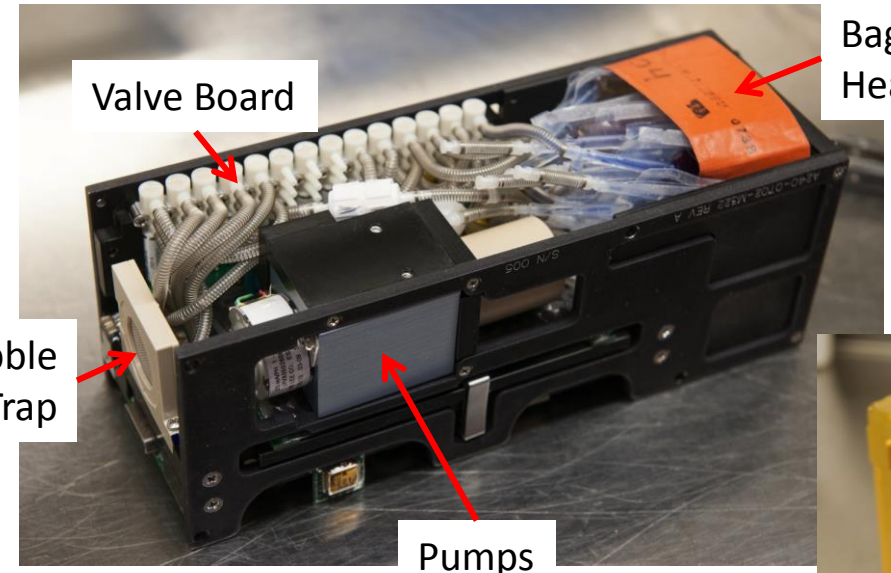
Fluidic System Schematic



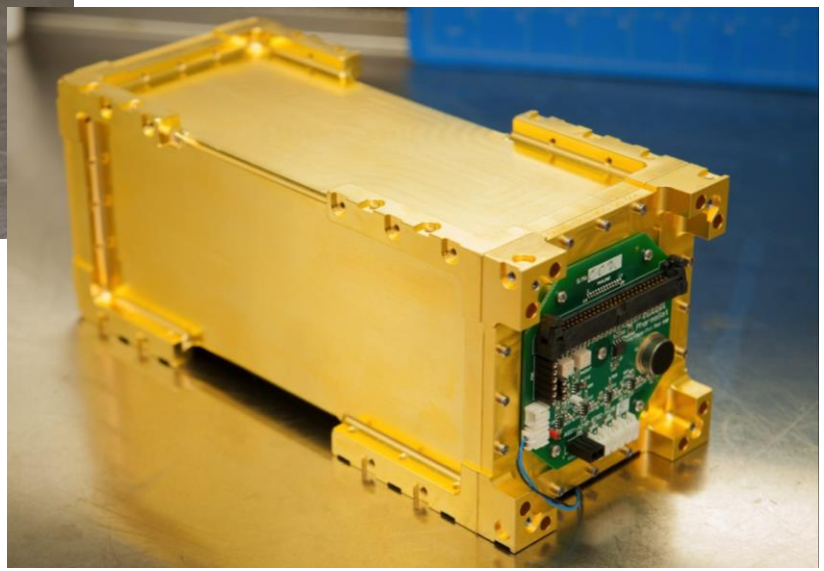
SV: Solenoid valve

Assembled Payload

Fluidic Payload



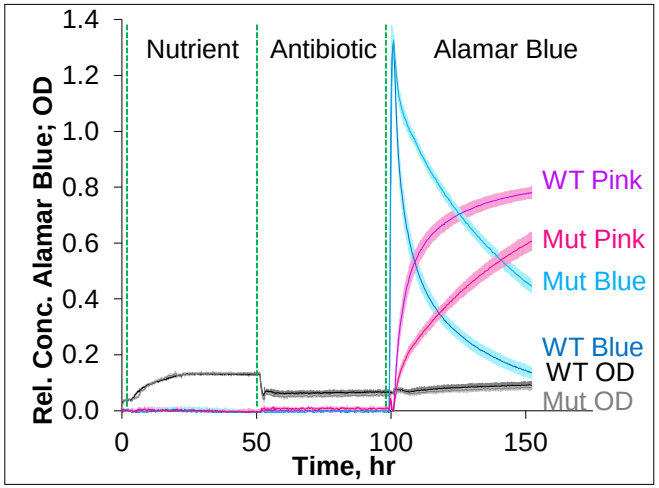
Hermetic Payload Can

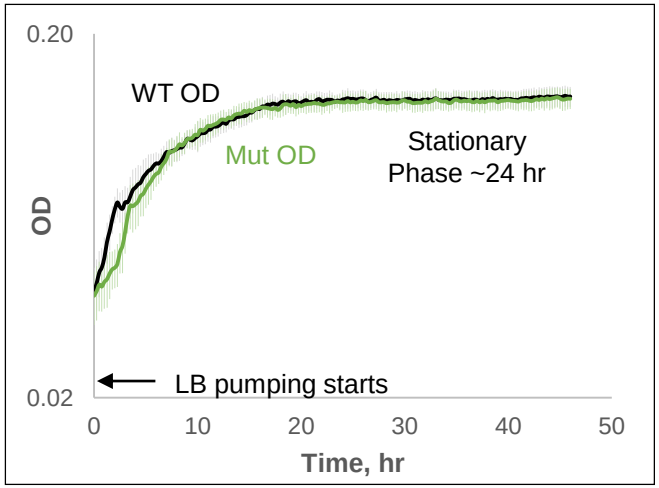


Can is 2U or roughly 10x10x20 cm

Experiment Verification on Ground

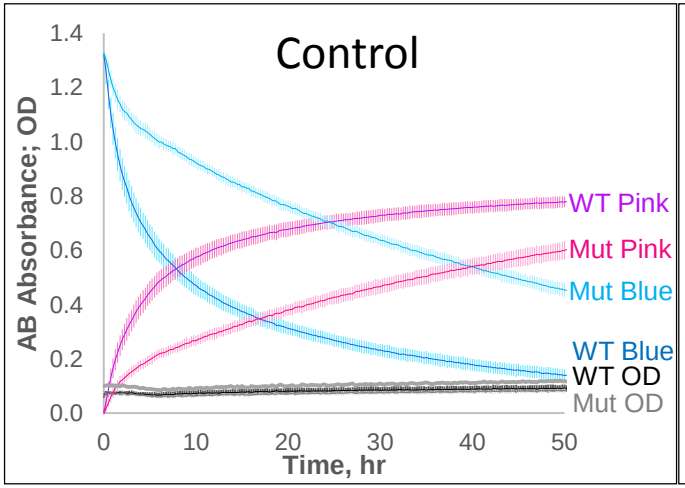
- Payload can rotated on rotisserie to prevent settling of cells
- Experiment run through “flight like” commanding
- Optics data calibrated and normalized
- Growth of wild type and mutant are similar

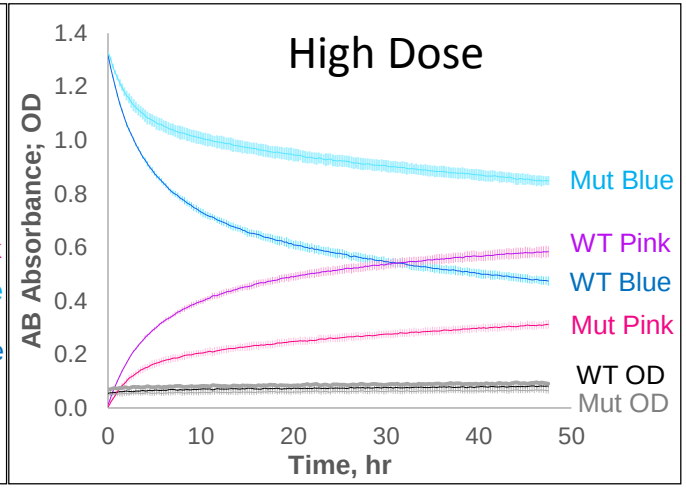


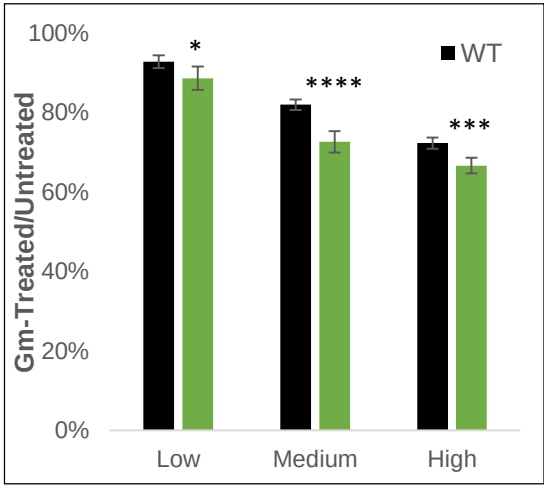


Evaluating the Effect of Gentamicin on *E. coli*

- Clear differences between wild type and mutant in control
- At each dose there was a significant difference in the ability of the cells to reduce alamarBlue relative to the control case







EcAMSat Summary

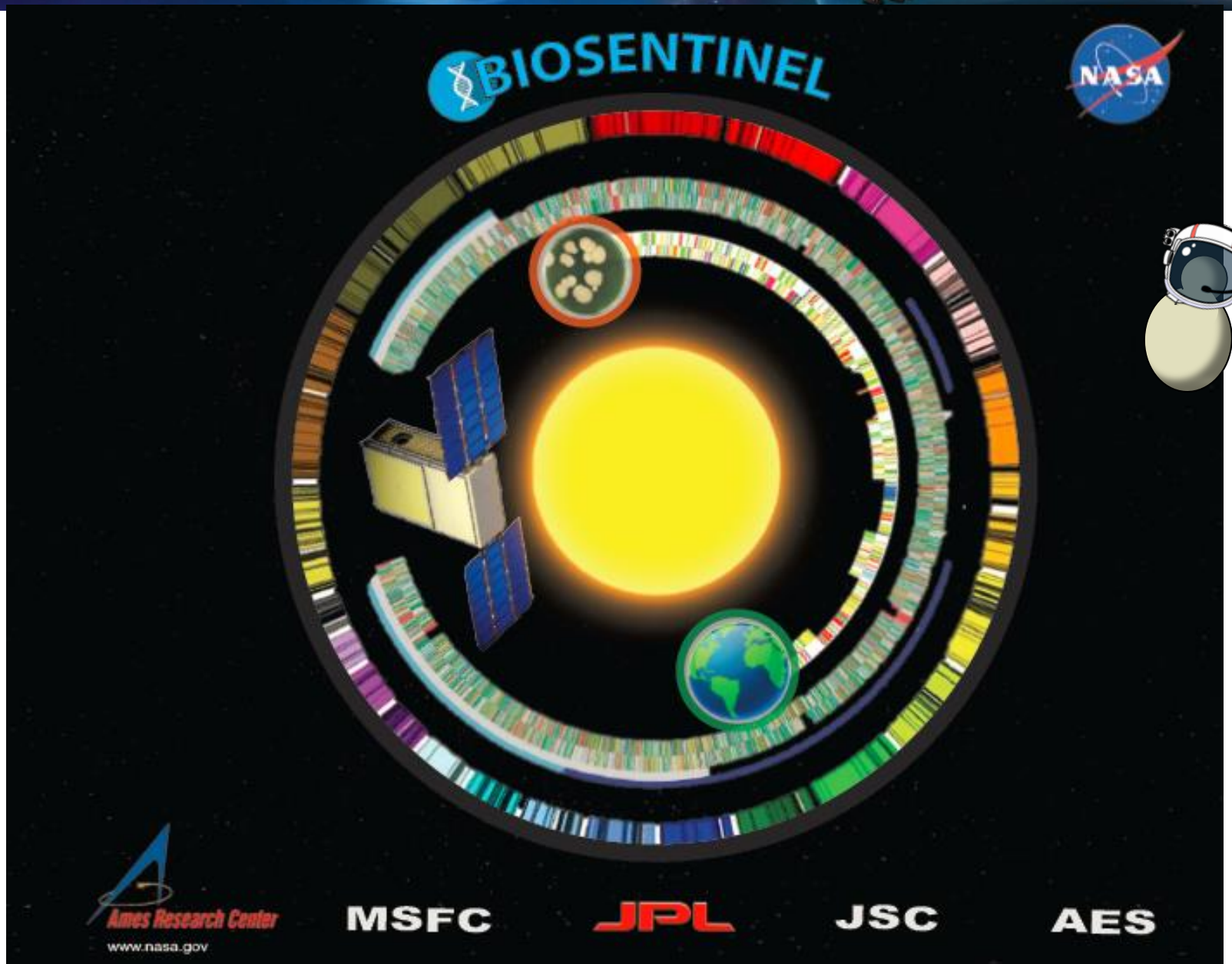
- EcAMSat leverages hardware from successful PharmaSat mission
  - Fluidic system provides accurate dilution of antibiotic and consistent delivery to the fluidics card housing the biology

- Uropathogenic *E. coli* model organism for studying changes in antibiotic resistance in microgravity
  - Extensive ground control tests already performed in addition to a 24 hr delayed synchronous ground control
  - Differences between WT and mutant strain missing key stress response gene, rpoS, can yield insight into mechanisms responsible for these changes
  - Help determine if therapies developed on ground will be successful in flight

Where are we now?

- Original scheduled launch: July 2015
  - Falcon 9 (SpaceX) FormoSat 5 + SHERPA
  - 6 launch delays
  - SHERPA de-manifested Jan 2017

- Current launch: Falcon 9 date TBD


BIOSENTINEL
NASA
Ames Research Center
www.nasa.gov
MSFC
JPL
JSC
AES

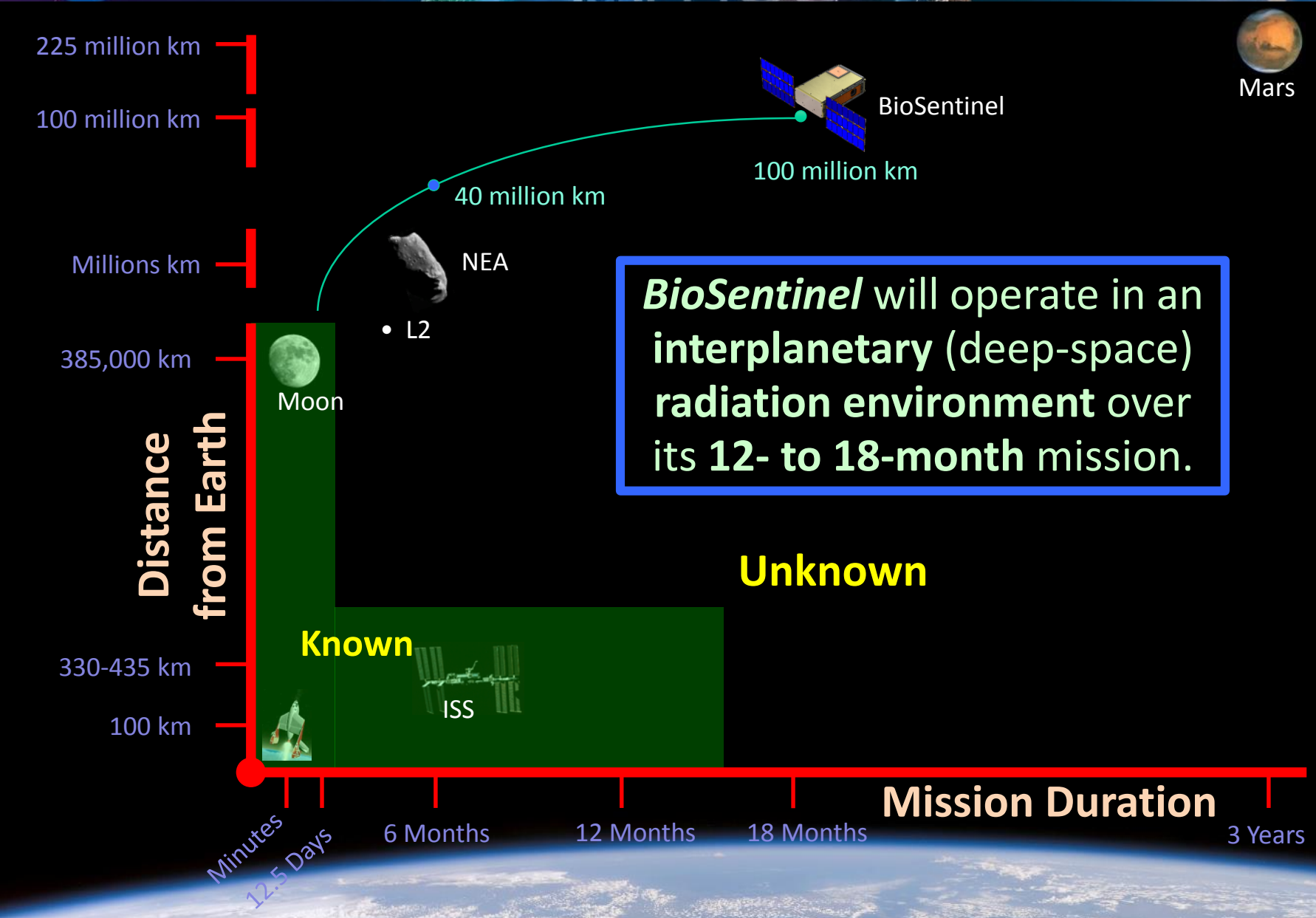
BioSentinel Team

- Bob Hanel, PM; Dawn McIntosh, Deputy PM
- Sharmila Bhattacharya, PI
- BioSensor Payload Team: Chetan Angadi, Josh Benton, Rich Bielawski, Justin Blaich, Travis Boone, James Chartres, Michael Dougherty, Lance Ellingson, Charlie Friedericks, Diana Gentry, Sarah Hayes, Mike Henschke, Liz Hyde, Ben Klamm, Diana Marina, Eddy Mazmanian, Griffin McCutcheon, Mike Padgen, Macarena Parra, Mario Perez, Abe Rademacher, Tony Ricco, Sergio Santa Maria, Aaron Schooley, Ann-Sofie Schreurs, Ming Tan, Eric Tapio, Huyen Tran
- NASA/Ames, NASA/JSC-Radworks, Loma Linda U. Med. Ctr., U. Saskatchewan
- Support from NASA's Advanced Exploration Systems and Human Exploration and Operations Mission Directorate

BioSentinel Space Radiation BioSensor Experiment & Mission

- BioSentinel is a 6U free-flying satellite to be delivered by NASA's Exploration Mission 1 (EM-1) to a heliocentric interplanetary orbit
  - First biology experiment beyond LEO since Apollo!

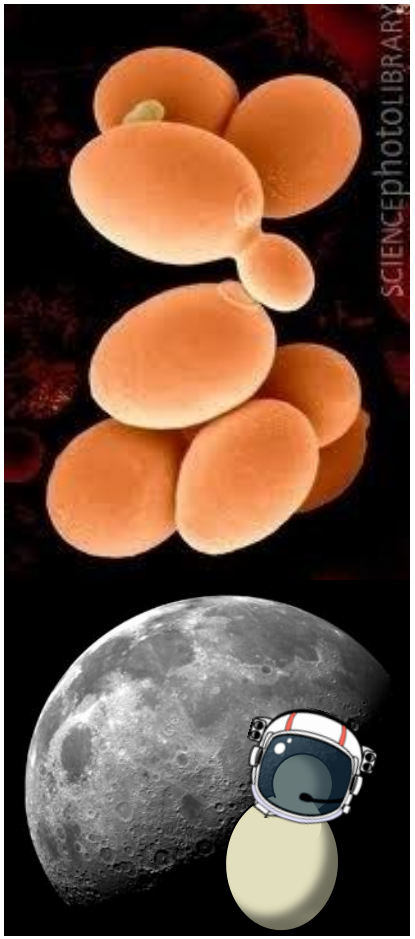
- Model organism, *S. cerevisae* (brewer's yeast) will be used to measure effects of deep space radiation on DNA damage
  - Help to understand, quantify, and potentially mitigate key biological risk



BioSentinel will operate in an **interplanetary** (deep-space) **radiation environment** over its **12- to 18-month** mission.

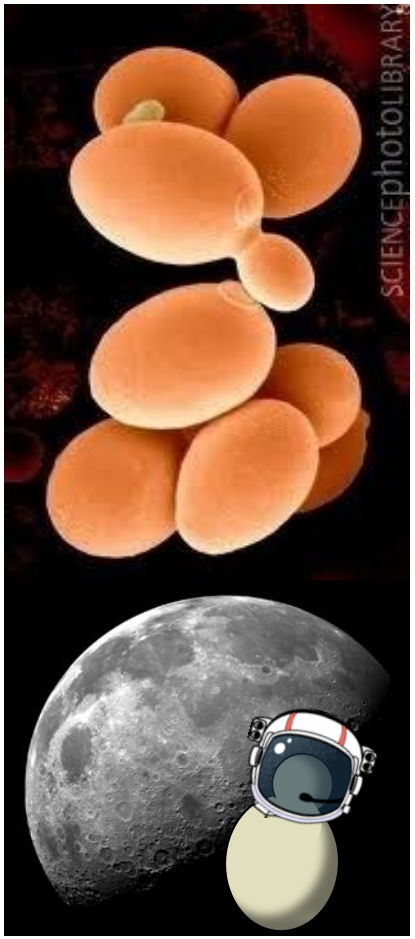
BioSentinel Science Mission: "*Canary in a Coal Mine*"

- Quantify DNA damage from space radiation environment
  - Space environment cannot be reproduced on Earth: *omnidirectional, continuous, low flux, variety of particle types*
  - Health risk for humans spending long durations beyond LEO
  - Radiation flux can spike 1000x during a solar particle event (SPE)
- Correlate biological response with physical radiation measurements
  - Linear Energy Transfer (LET) spectrometer bins and counts particle events by LET
  - Total Ionizing Dose (TID) sensor measures integrated deposited energy



BioSentinel Science Mission: "*Canary in a Coal Mine*"

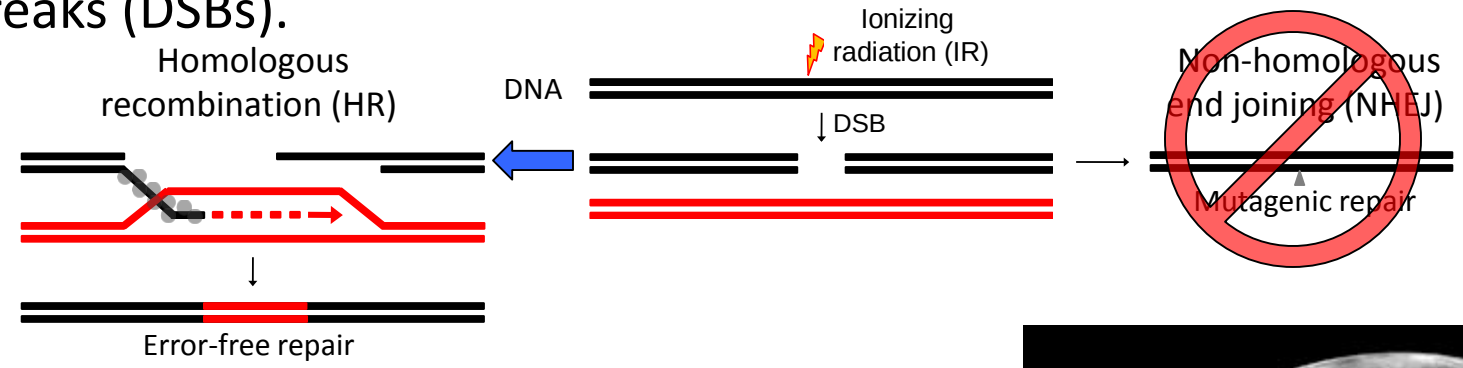
- Yeast assay: microfluidic arrays monitor DSB/repair
  - Three strains of *S. cerevisae*: 1 wild type, 1 radiation sensitve mutant, and 1 engineered strain
    - *engineered strain quantifies double strand breaks (DSBs)*
  - Wet and activate multiple banks of microwells over mission lifetime
  - Double strand break & associated repair enable cell growth & division
  - Reserve wells activated autonomously in case of SPE

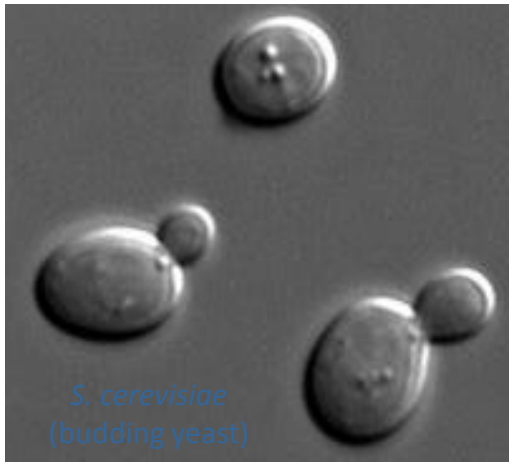


BioSensor DSB Detection Mechanism

BioSentinel: a Biosensor in Space

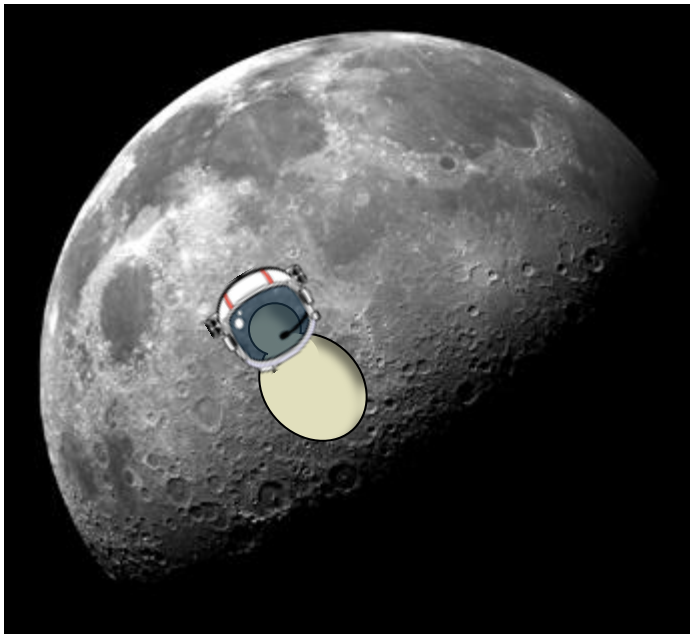
- What: BioSentinel is a yeast radiation biosensor that will measure the DNA damage caused by space radiation, specifically double strand breaks (DSBs).



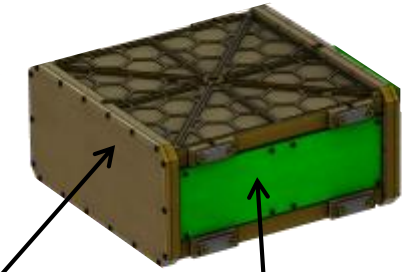


In yeast diploid cells, NHEJ is inhibited. Thus, HR is the main repair pathway for DSBs

One of our yeast strains is engineered in such a way that only the repaired cells will grow actively in the media provided and will be detected by optical density (OD) & colorimetric measurements



Spacecraft Configuration



Low-Gain Antenna

Command & Data Handling/Power/Transponder Stack

Propulsion System $(CF_3)_2CH_2$

Batteries

Integrated Guidance Navigation & Control Unit

Star Tracker Cutout

Medium-Gain Antenna

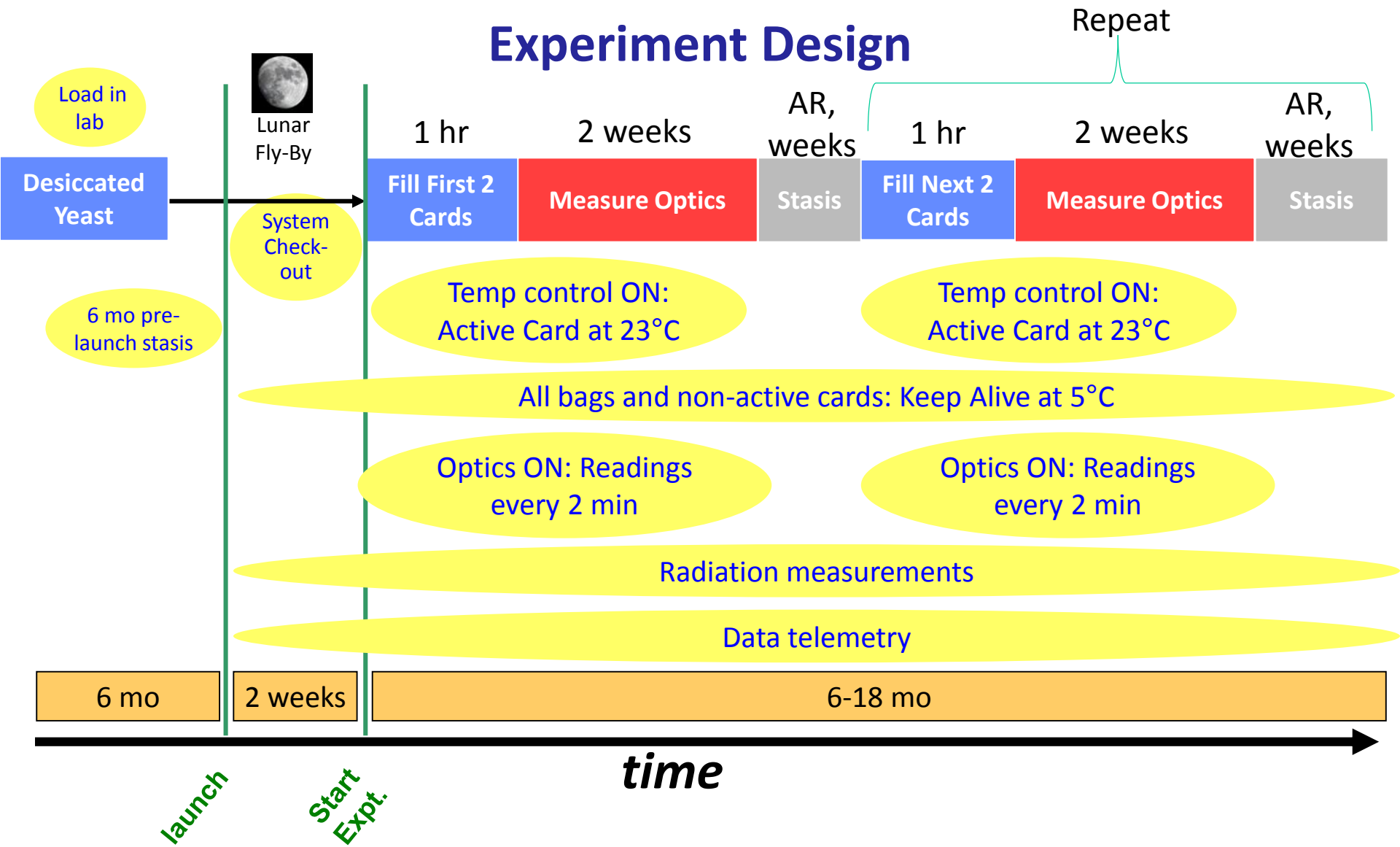
Radiation Sensors: TID + LET spectrometer

Solar Arrays

Solar Array Gimbal

Bio Payload Container (**4U, 4.5 kg**)

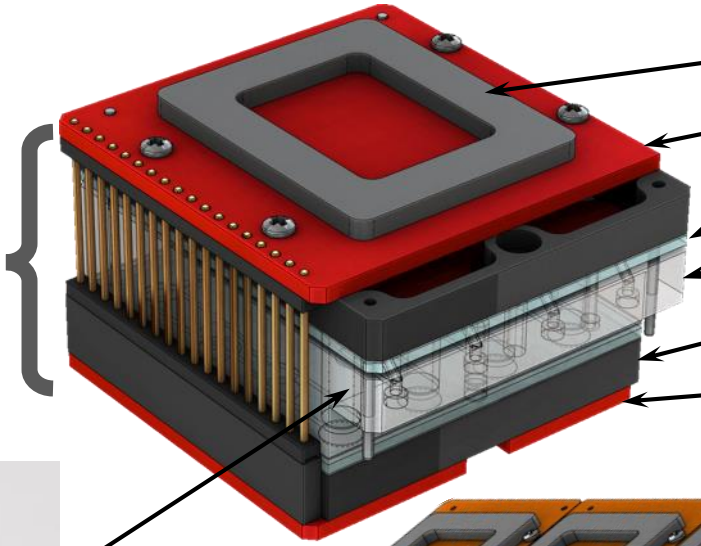
Experiment Design

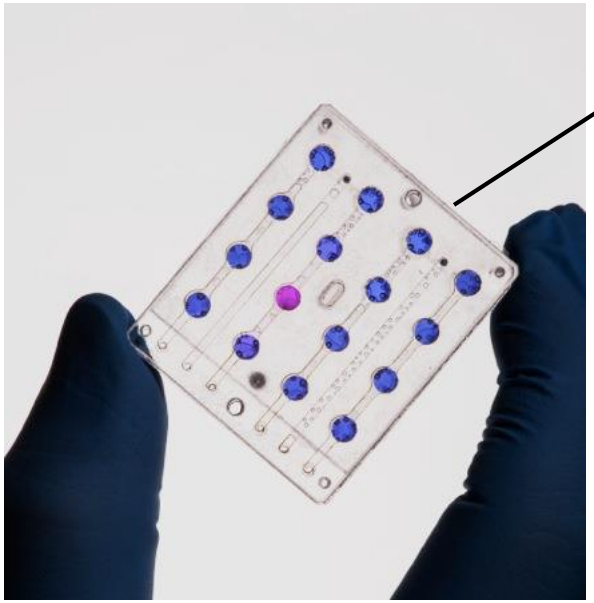


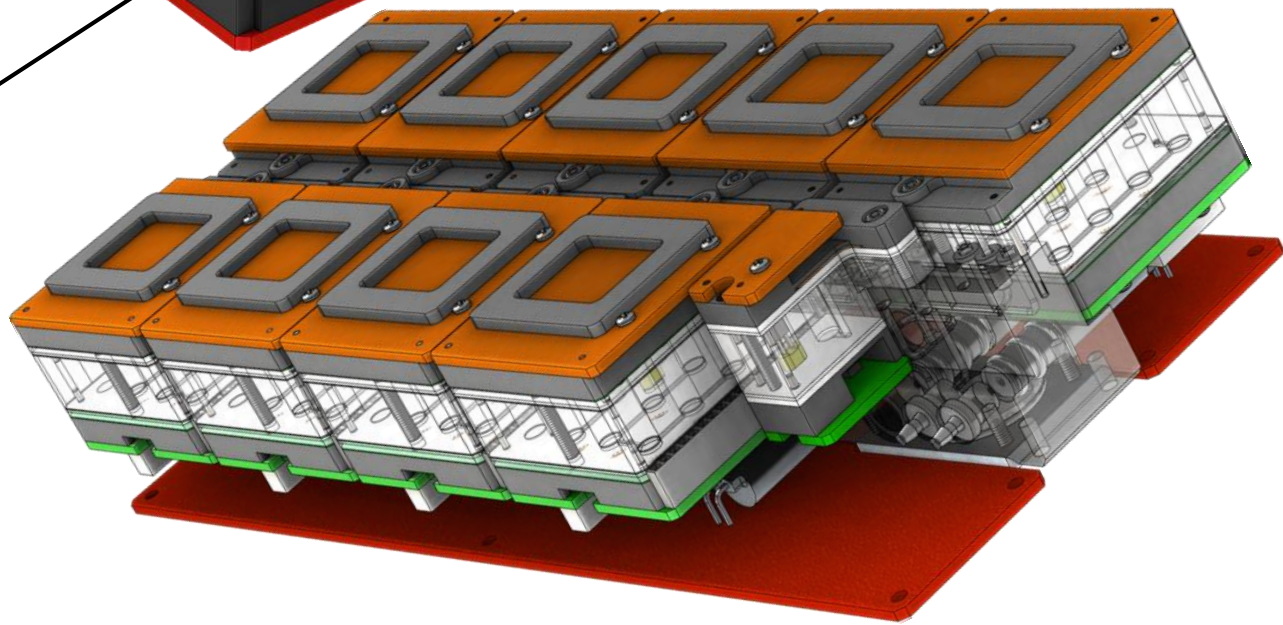
Note: A delayed synchronous ground control will be run

Bio-fluidic-opto-thermal Configuration

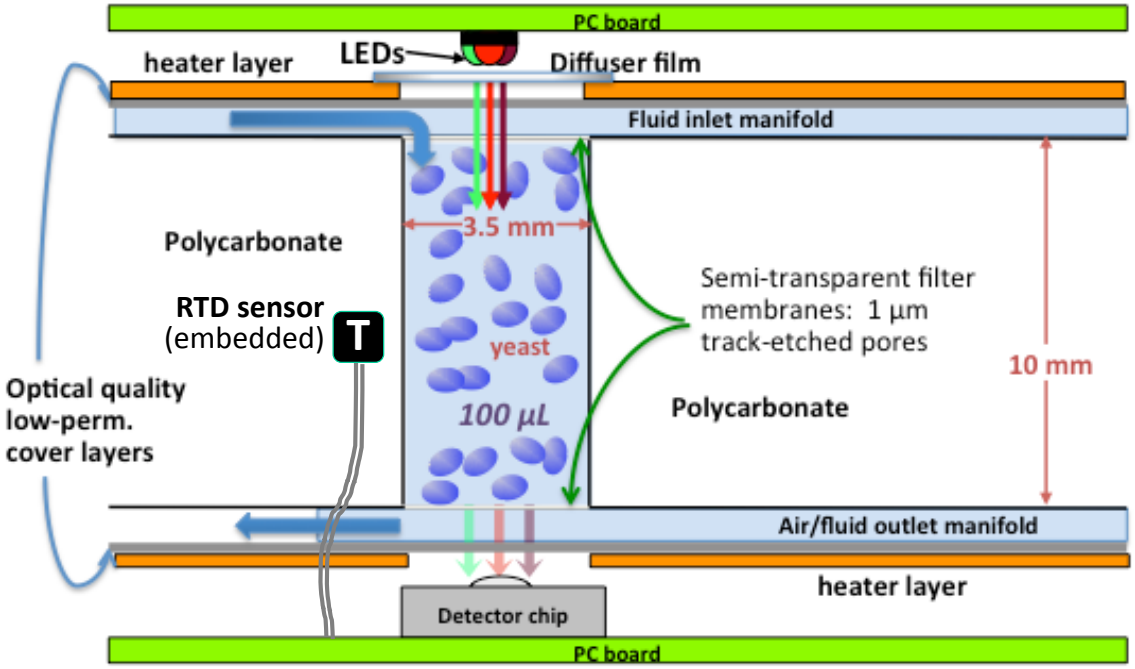
16-well card
= 1 "set"
(18 sets total)





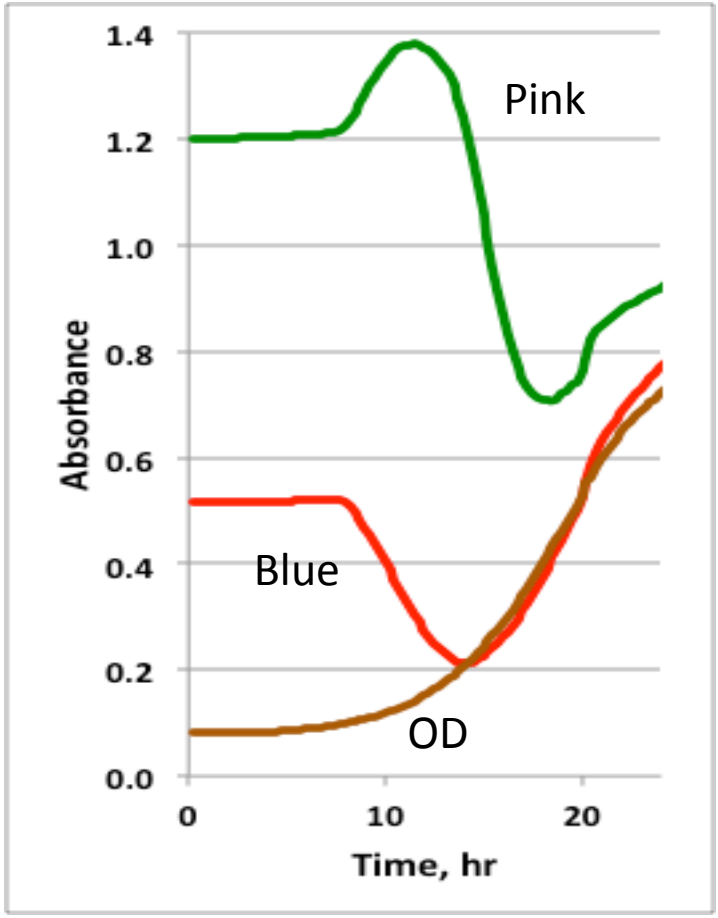


Bio-Fluidic-Opto-Thermal Cross Section

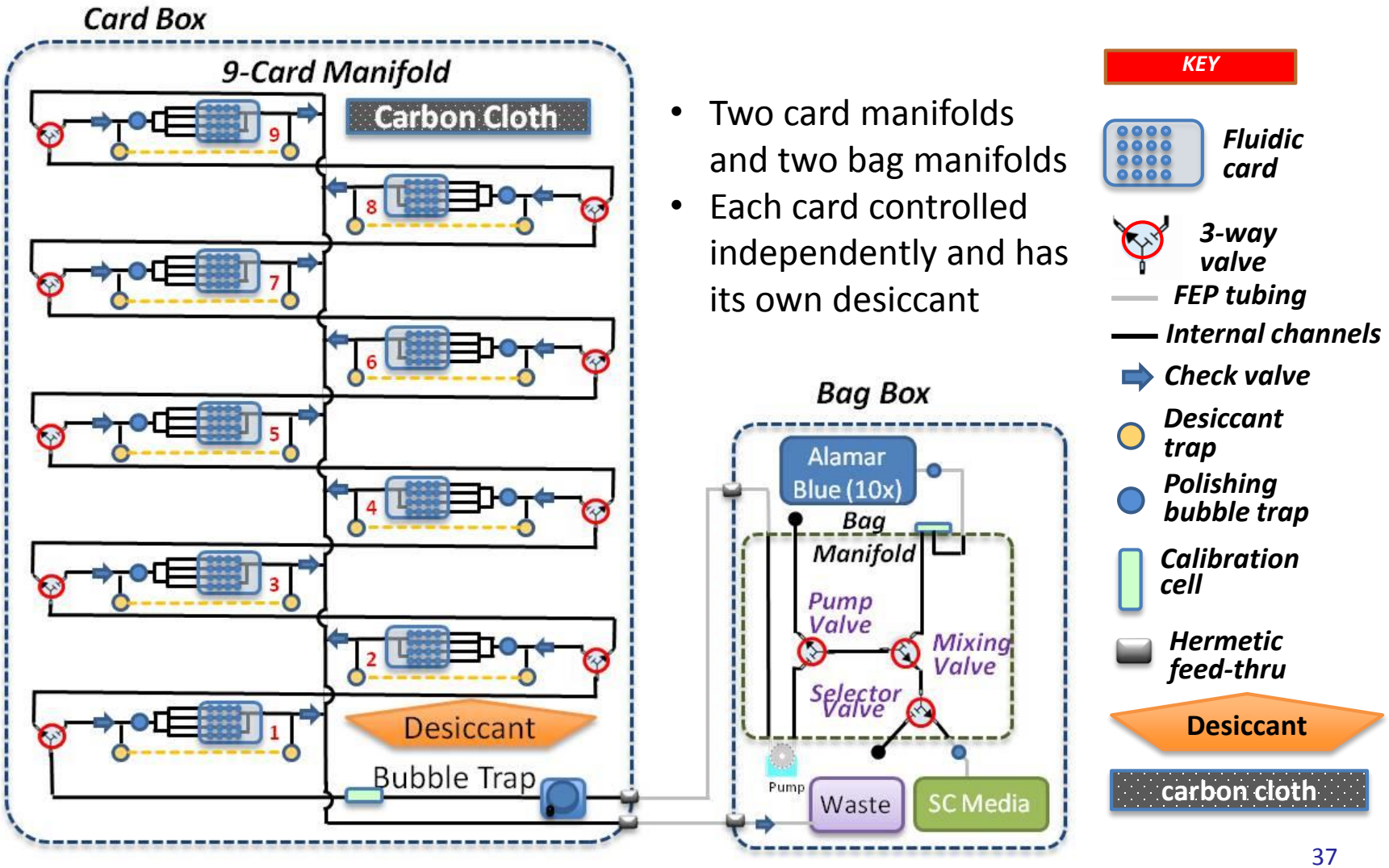


- Yeast dried onto µwell walls
- 3 LEDs + detector, per well, track growth *via* optical density and cell metabolic activity *via* dye color changes.
- LEDs: 570, 630, 850 nm

Yeast growth with flight-like prototype fluidic card + optics



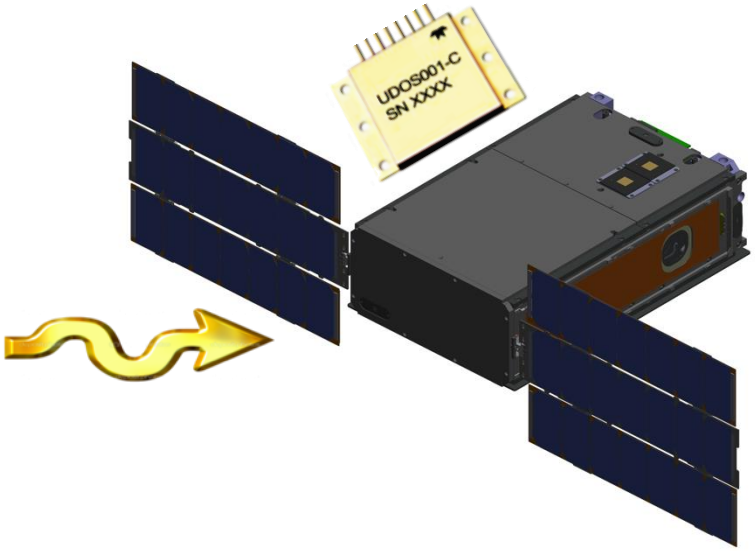
BioSentinel Payload: *Design/Function Highlights*

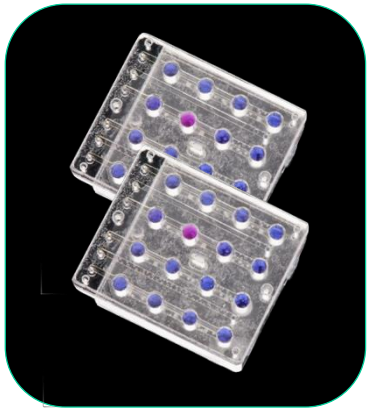


- Two card manifolds and two bag manifolds
- Each card controlled independently and has its own desiccant

SPE Autonomous Trigger for BioSensor

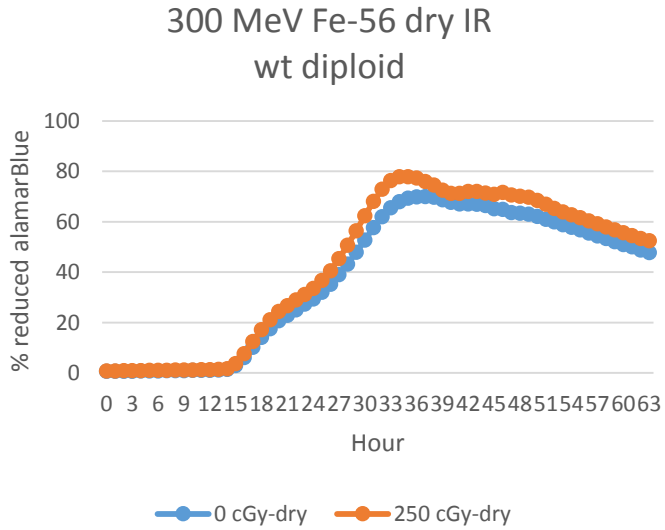
- Onboard TID monitor
- Ionizing EM radiation (gamma) precedes particles by ~ hours
  - SPE differentiable from smaller coronal mass ejections
- Activate “designated SPE set” of fluidic wells
  - measure radiation biological damage under wet conditions
  - *expected to generate more damage wet than in dry state* (OH•)

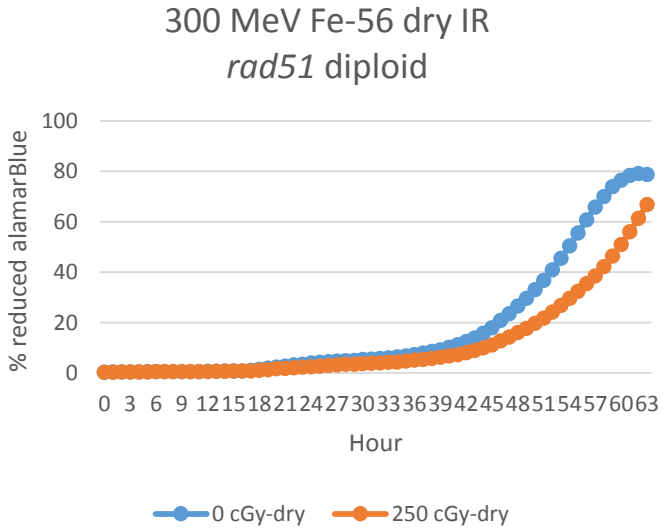




Radiation Sensitivity of Yeast Cells in Fluidics Cards

- Gamma, protons, iron, oxygen, silicon radiation sources have been used to quantify effects
- 2-year old air-dried cells are responsive to high-energy particle irradiation at 250 cGy (expected dose in a 12-month mission).





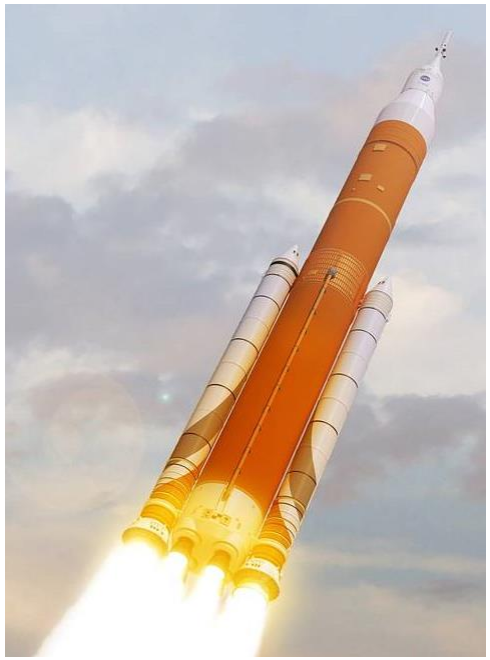
Summary of BioSentinel

- BioSentinel will be the first biology experiment outside of LEO since Apollo
- Heliocentric orbit will provide a deep space radiation environment for a 12 month mission
- *S. cerevisae* as a model organism will measure the effect of deep space radiation on DNA damage
  - Help to understand, quantify, and potentially mitigate key biological risk
- Demonstration of a deep space biosensor
  - Potential follow-on missions: Mars Sentinel, radiation shielding

Where are We Now?

- Building engineering development units for qualification and testing
- Long-term biocompatibility tests in progress
- Radiation testing, including SPE simulation

- Launch for SLS EM-1: Sept 30, 2018



https://www.nasa.gov/exploration/systems/sls/multimedia/images.html